

EXHIBIT A

Purpose: Test specificity of Myco Genus Probes Mch A 1026, Mch B 1634, & Mch B 1638 against rRNA's of a few possible cross-reacting organisms.

Procedure:

① Set up samples & controls in 280 μM PB lysis tubes, L/N 70123

a) 2 - 100 μl LNC, L/N 70224A

b) 2 - 100 μl *Trichomonas* rRNA, L/N 546-576-001

c) 2 - 1 μg *C. xerosis* rRNA, L/N 7064B

d) 2 - 1 μg *Mastigoides* rRNA, L/N 128-503-001

e) 2 - 1 μg *N. otitidis* rRNA, L/N # 7025

f) 2 - 1 μg *R. bronchialis* rRNA, L/N 616-572-001

② Add 1 ml ^{125}I -probe - Mch A 1026, Mch B 1634, & Mch B 1638

~15,000-20,000 cpm/ml (all ^{125}I) by M. Fluppe

③ Vortex, & add incubate at 72°C for 2 hrs

④ Add 4 ml 4% sepsoln (0.12M PB, 2% TGA), L/N 70147 to 10 tubes at a time on bench top.

⑤ Invert 80X, & incubate at 72°C for 5 min

⑥ Remove from bath, invert 20X, & spin at 2000 rpm for 2 min

⑦ Decant & blot

⑧ Add 4 ml wash (0.12M PB) L/N 70195

⑨ Vortex 20 seconds

⑩ Incubate at 72°C for 5 min.

⑪ Spin at 2,000 rpm for 2 min, decant, & blot

⑫ Count for 2 min

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Specificity - cont

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From Page No. 49 Results:

Mch A 1026

	<u>EWA</u>	<u>% Cypb</u>	<u>Neg Ratio</u>	<u>Cpm</u>	
Mtb	53.7	$53.6 > 53.4$	$37.7 > 37.6$	$9372 > 9333$	Total Counts = 700
Cxe	1.7	$1.5 > 1.2$	$1.2 > 0.9$	$294 > 216$	$17461 > 17462$
Nas	1.1	$1.4 > 1.6$	$0.8 > 1.1$	$188 > 284$	$17463 > 17462$
Not-cav	1.6	$1.6 > 1.5$	$1.1 > 1.1$	$272 > 262$	
Rbr	1.2	$1.3 > 1.4$	$0.9 > 1.0$	$215 > 244$	
LVC	1.4			$276 > 221$	
Mtb	71.3	$71.6 > 71.9$	$60.4 > 60.9$	$10985 > 11079$	Mch B 1634
Cxe	1.5	$1.4 > 1.3$	$1.2 > 1.1$	$225 > 196$	Total Counts = 15380 > 15426
Nas	6.8	$5.4 > 4.0$	$5.7 > 3.4$	$1042 > 612$	
Not-cav	6.9	$7.1 > 7.2$	$5.8 > 6.1$	$1060 > 1105$	
Rbr	3.8	$5.0 > 6.2$	$3.2 > 5.2$	$575 > 955$	
LVC	18	1.2		$184 > 180$	

See Book 678 for Mch B 1638 Results

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Witnessed & Understood by me,

NAT MUNOR

Date

Invented by

Dame J. Olden

Date

Recorded by